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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/089,211	03/25/2002	William E Hintz	2847-62447	4982

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EXAMINER	
STEADMAN, DAVID J	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 03/08/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/089,211	HINTZ ET AL.	
	Examiner	Art Unit	
	David J Steadman	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 January 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *3-7,10,11 and 20-30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3-7,10,11 and 20-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *03/25/02*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Status of the Application

[1] Claims 3-7, 10-11, and 20-30 are pending in the application.

[2] Applicants' amendment to the specification filed January 16, 2004 is acknowledged.

Lack of Unity

[3] Applicants' election with traverse of the invention of Group IX, original claims 3-7 and 10-11, drawn to the special technical feature of an isolated nucleic acid, a recombinant nucleic acid, a transformed cell, a transgenic fungus, and the first claimed method of use, *i.e.,* a method for producing a macromolecule having an altered glycosylation pattern, wherein the claims recite a nucleic acid encoding SEQ ID NO:18, including SEQ ID NO:17, filed January 16, 2004, is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Information Disclosure Statement

[4] All references cited by applicants in the information disclosure statement filed March 25, 2002 have been considered by the examiner with the exception of references EMBL Accession Number Q12563 and Herscovics et al. These references are not being considered by the examiner as copies of the references are not present in the file as

required by 37 CFR 1.98(a)(2), which requires a legible copy of each U.S. and foreign patent; each publication or that portion which caused it to be listed; and all other information or that portion which caused it to be listed. A copy of the information disclosure statement is attached to the instant Office action.

Specification/Informalities

[5] The use of the trademarks "Wizard™", "GigaPack™", "Genescreen Plus™", and "Bluescript™" (see pages 41-42) have been noted in this application. The trademarks cited above and any others present in the instant application should be capitalized wherever they appear and be accompanied by the generic terminology.

Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

[6] The specification discloses that SEQ ID NO:18 is the deduced amino acid sequence of SEQ ID NO:17. The specification is objected to as the nucleic acid of SEQ ID NO:17 encodes a polypeptide that does not correspond to the polypeptide of SEQ ID NO:18. A sequence alignment of SEQ ID NO:17 and SEQ ID NO:18 reveals that the codon "TAT" in SEQ ID NO:17 ", which encodes tyrosine, is translated as threonine (see Appendix A).

Priority

[7] Applicant's claim for domestic priority under 35 USC § 119(e) to provisional application 60/157,341, filed October 01, 1999, is acknowledged. The examiner can find no disclosure of the sequences of SEQ ID NO:17 and 18 in provisional application 60/157,341. In the absence of evidence to the contrary, applicant is granted ONLY the benefit of the earlier filing date of PCT/US00/27210, filed October 02, 2000, to the extent this application provides support for the claimed subject matter.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

[8] Claims 3-7, 10-11, and 20-30 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a specific and substantial asserted utility or well-established utility. The specification asserts the polypeptide of SEQ ID NO:18, encoded by the polynucleotide of SEQ ID NO:17 has utility for modifying the glycosylation pattern of proteins *in vitro* and *in vivo* (see pages 27-28 of the instant specification). However, this asserted utility is not specific and substantial. Mannosidases comprise a highly diverse group of enzymes having a variety of enzymatic activities (see Appendix C). Even the more specific term alpha-1,2-mannosidase encompasses at least 2 different enzymatic activities (see 1) and 3) of Appendix C). The specification fails to disclose the specific enzymatic activity and particular substrate of the polypeptide of SEQ ID NO:18, encoded by SEQ ID NO:17.

Eades et al. (*Gene* 255:25-34), who describe the isolation of a protein having 100% sequence identity to SEQ ID NO:18, fails to identify the substrate of their isolated polypeptide and teaches that further experimentation is required for such identification by teaching, "[p]urification of the *A. nidulans* alpha-1,2-mannosidases and determination of their substrate specificities will clarify their role in *N*-glycan processing" (page 33, left column, middle). Eades et al. further teaches that "the engineering of in vivo processing of N-glycans from lower eukaryotes to the complex N-glycans of higher eukaryotes requires... ...a suitable substrate (i.e. $Man_5GlcNAc_2$) upon which GnT-I can act. The production of a suitable substrate for GnT-I activity may be more problematic, as there may be a 'bottleneck' preventing the production of significant amounts of $Man_5GlcNAc_2$." (page 33, left column, middle). However, further experimentation is required in order to determine whether overexpression of the polypeptide of SEQ ID NO:18 will relieve such a 'bottleneck' as evidenced by Eades et al. who teach, "controlled overexpression of the three Class I alpha-mannosidases may clear the 'bottleneck' and allow production of complex *N*-glycans" (page 33, left column, bottom; underline added for emphasis). In order to use the polypeptide of SEQ ID NO:18 for modifying the glycosylation pattern of a protein and in particular to generate the substrate required for complex N-glycan processing, *i.e.,* $Man_5GlcNAc_2$, one of ordinary skill in the art would necessarily need to know the specific alpha-1,2-mannosidase activity, the substrate specificity of SEQ ID NO:18, and the product generated by the polypeptide of SEQ ID NO:18. However, there is no evidence of record that such information and guidance was present in the specification or the prior art of record. As such, one of ordinary skill in the art would

recognize that further experimentation is required for a "real world" use of the claimed nucleic acid and method. This type of utility is not considered a "substantial utility". See e.g., *Brenner v. Manson,* 383 U.S. 519, 148 USPQ 689 (Sup. Ct. 1966). The specification must teach a skilled artisan how to use what is claimed and not merely provide a blueprint for further experimentation in order for an artisan to identify a use for the claimed invention. As stated in *Brenner v. Manson*, 383 U.S. 519 535-536, 148 USPQ 689, 696 (1966), "[a] patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion". Here the specification fails to provide a specific benefit in currently available form for the claimed nucleic acid as additional research is required as evidenced by Eades et al. in order to use the nucleic acid according to the asserted utility as set forth in the specification.

Claim Rejections - 35 USC § 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

[9] Claim(s) 3-7 and 10 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

[a] Claim 3 (claims 4-7 and 10 dependent therefrom) is confusing because, as written, the claimed nucleic acid is required to simultaneously have all three limitations as set forth in parts a)-c) of the claim. It is suggested that, for example, "and" at line 5 be replaced with "or" OR the claim be amended to insert "an amino acid sequence

selected from the group consisting of" following "wherein the protein comprises". See MPEP 2111.03 regarding transitional phrases.

[b] Claim 10 recites the limitation "the transformed cell of claim 4". There is insufficient antecedent basis for this limitation in the claim. It appears claim 10 should depend from claim 5 and it has been examined accordingly.

Claim Rejections - 35 USC § 112, First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

[10] Claims 3-7, 10-11, 20-26, and 28-29 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of he claimed invention.

Claims 3 (claims 4-7 dependent therefrom), 10-11, 20-26, and 28-29 are drawn to a genus of isolated nucleic acids encoding variants of SEQ ID NO:18, a genus of isolated nucleic acid variants and fragments of SEQ ID NO:17, or a method for producing a genus of macromolecules having an altered glycosylation pattern.

For claims drawn to a genus, MPEP § 2163 states the written description requirement for a claimed genus may be satisfied through sufficient description of a

representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. Regarding the claimed genus of nucleic acids of claims 3-7, 11, 20-26, and 28-29, the specification discloses only a SINGLE representative species of the claimed genus of nucleic acids, *i.e.,* SEQ ID NO:17, encoding SEQ ID NO:18, which has alpha-1,2-mannosidase activity. The specification fails to describe any additional representative species of the claimed genus. While MPEP § 2163 acknowledges that in certain situations "one species adequately supports a genus", it is also acknowledges that "[f]or inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus". In the instant case, the claimed genus of nucleic acids encompasses species that are widely variant in both structure and function, including (but not limited to) genomic sequences, allelic variants, and nucleic acid variants encoding polypeptides having function other than the alpha-1,2-mannosidase activity of SEQ ID NO:18, *e.g.,* non-functional polypeptides and

polypeptides having activity other than the asserted alpha-1,2-mannosidase activity, including the numerous mannosidase activities known in the art (see Appendix C). As such, the disclosure of the single representative species of SEQ ID NO:17 is insufficient to be representative of the attributes and features of *all* species encompassed by the claimed genus of nucleic acids. Regarding the genus of recited macromolecules having an altered glycosylation pattern as made by the method of claim 10, as noted above, this genus is widely variant with respect to both structure and function. Again the specification discloses only a SINGLE representative species of the claimed genus of macromolecules having an altered glycosylation pattern, *i.e.,* a method for releasing mannose from the substrate mannose-alpha-1,2-mannose-alpha-O-CH_3. Given the lack of description of a representative number of polynucleotides, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicant was in possession of the claimed invention.

[11] Claims 3-7, 10-11, and 20-30 are rejected under 35 U.S.C. 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention.

[12] Even if a polynucleotide encoding SEQ ID NO:18 is found to have patentable utility, the following rejection still applies: claim(s) 3-7, 10-11, 20-26, and 28-29 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for an isolated nucleic acid encoding SEQ ID NO:18 and a method for

releasing mannose from the substrate mannose-alpha-1,2-mannose-alpha-O-CH_3, does not reasonably provide enablement for all isolated nucleic acids encoding variants of SEQ ID NO:18 or all isolated nucleic acid variants and fragments of SEQ ID NO:17 as encompassed by the claims, or all macromolecules having an altered glycosylation pattern as produced by the method of claim 10. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

It is the examiner's position that undue experimentation would be required for a skilled artisan to make and/or use the entire scope of the claimed invention. Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See MPEP § 2164.01(a). The Factors most relevant to the instant rejection are addressed in detail below.

- The claims are overly broad in scope: The claims are so broad as to encompass all isolated nucleic acids encoding variants of SEQ ID NO:18 or all isolated nucleic acid variants and fragments of SEQ ID NO:17 as encompassed by the claims, or all macromolecules having an altered glycosylation pattern as produced by the method of

claim 10. The broad scope of claimed nucleic acids or recited macromolecules with an altered glycosylation pattern are not commensurate with the enablement provided by the disclosure with regard to the extremely large number of nucleic acids and macromolecules broadly encompassed by the claims. In this case the disclosure is limited to an isolated nucleic acid encoding SEQ ID NO:18 and a method for releasing mannose from the substrate mannose-alpha-1,2-mannose-alpha-O-CH_3.

- The lack of guidance and working examples: The specification provides only a single working example of the claimed nucleic acids, *i.e.,* SEQ ID NO:17, encoding SEQ ID NO:18 having alpha-1,2-mannosidase enzymatic activity and the specification provides only a single working example of the claimed method for producing a macromolecule having an altered glycosylation pattern, *i.e.,* a method for releasing mannose from the substrate mannose-alpha-1,2-mannose-alpha-O-CH_3. These working examples fail to provide the necessary guidance for making and/or using the entire scope of claimed nucleic acids or methods. Regarding the claimed nucleic acids, the specification fails to provide guidance regarding those nucleotides of SEQ ID NO:17 or amino acids of SEQ ID NO:18 that may be altered by substitution, addition, insertion, and/or deletion with an expectation of maintaining the desired alpha-1,2-mannosidase activity. Furthermore, the specification fails to provide guidance as to how to use those variant nucleic acids that encode polypeptides having activities other than the desired activity, *e.g.*, nucleic acids encoding non-functional polypeptides or polypeptides having activity other than the asserted alpha-1,2-mannosidase activity, including the numerous mannosidase activities known in the art (see Appendix C). Furthermore, the

specification fails to provide guidance for using those expressed variants of SEQ ID NO:18 having no activity or activity other than the asserted alpha-1,2-mannosidase activity to alter the glycosylation pattern of a macromolecule.

- The high level of unpredictability in the art: The nucleotide sequence of an encoding nucleic acid determines the corresponding encoded protein's structural and functional properties. Predictability of which changes can be tolerated in an encoded protein's amino acid sequence – conservative or non-conservative amino acid changes – and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e., expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. The positions within an encoding nucleic acid's sequence where modifications can be made with a reasonable expectation of success in obtaining an encoded polypeptide having the desired activity/utility are limited in any protein and the result of such modifications is HIGHLY unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, *e.g.,* multiple substitutions. In this case, the necessary guidance has not been provided in the specification as explained in detail above. Thus, a skilled artisan would recognize the high level of unpredictability that the entire scope of nucleic acids would encode a polypeptide having the desired activity. As the claimed nucleic acid encoding variants of SEQ ID NO:18 may or may not encode polypeptides having the desired activity – there

is no way to predict the effect(s) of such modification(s) – it is highly unpredictable as to whether the expressed variant can be used to practice the method of claim 10.

- The state of the prior art supports the high degree of unpredictability: The state of the art provides evidence for the high degree of unpredictability in altering a polynucleotide sequence with an expectation that the encoded polypeptide will maintain the desired activity/utility. For example, Branden et al. ("Introduction to Protein Structure", Garland Publishing Inc., New York, 1991) teach "[p]rotein engineers frequently have been surprised by the range of effects caused by single mutations that they hoped would change only one specific and simple property in enzymes" and "[t]he often surprising results of such experiments reveal how little we know about the rules of protein stability... ...they also serve to emphasize how difficult it is to design *de novo* stable proteins with specific functions" (page 247). While it is acknowledged that this reference was published in 1991, to date there remains no certain method for reasonably predicting the effects of even a *single* amino acid mutation on a protein.

- The amount of experimentation required is undue: While methods of generating variants of a given polynucleotide are known, *e.g.,* site-directed or random mutagenesis, and methods of isolating homologous polynucleotides are known, *e.g.,* hybridization, it is not routine in the art to screen for *all* nucleic acid variants and fragments having a substantial number of modifications and encoding polypeptides having a broad range of functions, as encompassed by the instant claims. In view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, and the high degree of unpredictability as evidenced by the prior art, undue

experimentation is necessary for a skilled artisan to make and use the entire scope of the claimed invention.

Thus, applicant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

[13] Claim(s) 11, 20-23, and 28 are rejected under 35 U.S.C. 102(b) as being anticipated by Database GenBank Accession Number AA965900 (GI:3139784). The claims are drawn to an isolated nucleic acid comprising at least 15, 20, 30, 40, or 50 contiguous nucleotides of SEQ ID NO:17 (claims 11 and 20-23) or an isolated nucleic

acid comprising a sequence that can hybridize to SEQ ID NO:17 under the conditions set forth in claim 28. GenBank Accession Number AA965900 discloses the sequence of an isolated nucleic acid that is 100% identical to nucleotides 371 to 894 of SEQ ID NO:17 (see Appendix B). This anticipates claims 11, 20-23, and 28 as written.

Conclusion

[14] Status of the claims:

- Claims 3-7, 10-11, and 20-30 are pending.
- Claims 3-7, 10-11, and 20-30 are rejected.
- No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (571) 272-0942. The Examiner can normally be reached Monday-Friday from 7:00 am to 5:00 pm. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (571) 272-0928. The FAX number for submission of official papers to Group 1600 is (703) 308-4242. Draft or informal FAX communications should be directed to (571) 273-0942. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.
Patent Examiner
Art Unit 1652

03-05-04

APPENDIX A (Alignment of SEQ ID NO:17 and 18)

us-10-089-211-18 (1-586) x us-10-089-211-17 (1-2032)

```
Qy          1 MetProArgArgTrpSerSerLeuIleSerIleThrAlaIlePheLeuValLeuPhePhe 20
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         36 ATGCCGAGACGGTGGTCCTCCCTCATCAGCATCACAGCCATCTTCTTGGTCCTCTTCTTC 95

Qy         21 LeuLeuHisArgAsnThrAspThrProArgAlaAlaAsnArgAlaThrAsnGlyProAla 40
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         96 CTCCTTCATAGGAATACAGACACACCACGCGCCGCCAATAGGGCTACAAACGGCCCTGCC 155

Qy         41 AsnGlyPheAlaArgGlnGlnSerIleCysProSerThrProProGlnProProThrAsn 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||   |||
Db        156 AACGGCTTTGCTAGGCAGCAAAGCATATGTCCATCAACACCCCCTCAGCCTCCATATAAC 215

Qy         61 ArgThrSerThrGlyGlyPheAsnTrpGlyGluIleProValArgThrProValSerAsp 80
              ||||||||||||||||||||||||||||||||||||||||||||   ||||||||||||
Db        216 CGAACCAGCACGGGAGGGTTCAACTGGGGTGAAATCCCAGTCAGATACCCTGTATCCGAC 275

Qy         81 PheIleProLeuSerThrAsnSerProAlaThrLeuProArgIleGlnArgSerSerPhe 100
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        276 TTCATCCCGCTGTCAACCAACTCTCCTGCAACACTTCCGCGCATCCAACGCTCTTCCTTC 335

Qy        101 ProLeuGlnSerSerIleThrLysSerArgGlnAlaAlaValLysGlyAlaPheGlnArg 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        336 CCACTTCAATCCTCAATCACTAAATCCCGCCAGGCAGCAGTCAAAGGTGCCTTTCAGCGC 395

Qy        121 AlaTrpThrSerThrThrThrHisAlaTrpLysAlaAspGluValArgProIleThrAla 140
              ||||||||||||   ||||||||||||||||||||||||||||||||||||||||||||||
Db        396 GCATGGACCTCCTACACAACCCACGCCTGGAAGGCGGACGAGGTACGGCCCATCACGGCC 455

Qy        141 GlySerArgAsnAsnPheGlyGlyTrpGlyAlaThrLeuValAspAsnLeuAspThrLeu 160
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        456 GGATCTCGAAACAACTTTGGCGGATGGGGAGCGACCCTAGTCGACAATCTCGACACACTG 515

Qy        161 LeuIleMetGlyLeuAspGluGluPheAlaAlaAlaValAspAlaLeuAlaAspIleGlu 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        516 CTAATCATGGGGCTGGACGAGGAGTTCGCAGCGGCAGTCGACGCGCTCGCAGATATAGAA 575

Qy        181 PheSerProHisSerSerProSerSerSerGlnSerThrIleAsnIlePheGluThrThr 200
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        576 TTCAGCCCGCACTCGTCCCCATCCTCCTCCCAGAGCACAATCAACATATTCGAAACGACA 635

Qy        201 IleArgThrLeuGlyGlyLeuLeuAlaAlaThrAspLeuThrGlyCysArgGluThrArg 220
              ||||||   ||||||||||||||||||||   ||||||||||||||||||||||||||||
Db        636 ATCCGGTATCTGGGCGGCTTGCTCGCGGCGTATGATCTCACTGGCTGTCGAGAGACTCGG 695

Qy        221 LeuLeuAspLysAlaIleGlnLeuGlyGluMetIleThrThrSerPheAspThrGluAsn 240
              ||||||||||||||||||||||||||||||||||   ||||||||||||||||||||||
Db        696 CTGCTGGACAAAGCAATCCAGCTTGGGGAGATGATCTACACCTCCTTCGACACAGAGAAC 755

Qy        241 ArgMetProValProArgTrpAsnLeuHisLysAlaGlyAsnGlyGluProGlnArgAla 260
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        756 CGCATGCCCGTACCACGGTGGAATCTGCACAAAGCAGGCAACGGAGAGCCTCAGCGCGCG 815

Qy        261 AlaValGlnGlyValLeuAlaGluLeuAlaSerSerSerLeuGluPheThrArgLeuSer 280
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        816 GCAGTGCAGGGCGTGCTCGCTGAACTCGCCAGCAGCAGTCTCGAGTTCACGCGGCTGTCG 875

Qy        281 GlnLeuThrGlyAspMetArgThrPheAspAlaAlaSerArgIleThrAspLeuLeuAsp 300
              ||||||||||||||||||||   ||||||||||||||||||||||||||||||||||||
Db        876 CAGCTGACGGGGGATATGCGGTATTTCGATGCGGCATCCCGCATTACCGATCTGCTTGAC 935

Qy        301 SerGlnAlaGlyHisThrArgIleProGlyLeuTrpProValSerValAsnLeuGlnLys 320
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        936 TCCCAAGCCGGCCATACCCGGATCCCGGGGTTGTGGCCAGTCAGCGTGAACCTGCAGAAA 995
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Qy        321 GlyAspLeuThrArgGlySerThrPheSerPheGlyGlyMetAlaAspSerAlaThrGlu 340
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||   |||
Db        996 GGCGATCTGACCCGTGGGTCGACATTCAGTTTTGGCGGGATGGCCGATAGCGCCTACGAG 1055

Qy        341 ThrLeuGlyLysThrThrArgLeuLeuGlyGlyValGlyLysGlyProGlnThrGluArg 360
                 ||||||||||||   ||||||||||||||||||||||||||||||||||||   ||||||
Db       1056 TATCTCGGCAAGACGTATCGGCTCCTCGGTGGTGTGGGGAAAGGGCCACAGTACGAGCGT 1115

Qy        361 LeuAlaArgAsnAlaLeuAspAlaGlyIleArgHisLeuLeuPheArgProMetThrPro 380
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1116 CTGGCGCGAAACGCACTAGATGCCGGGATTCGACATCTCCTCTTCCGACCGATGACGCCT 1175

Qy        381 AspHisAlaAspIleLeuLeuProGlyValAlaHisAlaThrSerSerSerValGlyLeu 400
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1176 GATCATGCAGATATCCTCCTACCCGGGGTCGCGCACGCAACCAGCTCTTCCGTGGGACTC 1235

Qy        401 GluProArgThrGluHisLeuAlaCysPheValGlyGlyMetThrAlaLeuAlaGlyLys 420
              ||||||||||||||||||||||||||||||||||||||||||   ||||||||||||||||
Db       1236 GAGCCCCGGACAGAGCATCTCGCCTGTTTTGTGGGTGGGATGTACGCGCTCGCCGGGAAG 1295

Qy        421 LeuPheSerAsnGlnThrThrLeuAspThrGlyArgLysLeuThrAspGlyCysIleTrp 440
              ||||||||||||||||||   ||||||||||||||||||||||||||||||||||||||||
Db       1296 CTTTTCTCAAACCAGACGTACCTCGACACCGGCCGGAAGCTGACAGACGGTTGTATCTGG 1355

Qy        441 ThrThrAspAsnSerProLeuGlyIleMetProGluMetPheThrValProAlaCysPro 460
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1356 TACTACGATAATTCACCGCTAGGTATCATGCCGGAGATGTTCACCGTGCCGGCTTGTCCG 1415

Qy        461 SerValAlaGluCysProTrpAspGluThrArgGlyGlyIleThrThrThrValArgAsp 480
              ||||||||||||||||||||||||||||||||||||||||   |||   |||||||||
Db       1416 TCAGTGGCTGAATGTCCTTGGGACGAAACAAGGGGTGGTATCTACACCTACGTGCGTGAT 1475

Qy        481 GlyHisThrPheLeuArgProGluAlaMetGluSerIlePheThrMetTrpArgIleThr 500
              ||||||   ||||||||||||||||||||||||||||||||   ||||||||||||||||
Db       1476 GGGCACTACTTTCTGCGTCCTGAGGCAATGGAGAGTATCTTCTATATGTGGCGCATTACA 1535

Qy        501 GlyAspGluLysThrArgGluAlaAlaTrpArgMetPheThrAlaIleGluAlaValThr 520
              ||||||||||||   ||||||||||||||||||||||||||||||||||||||||||||||
Db       1536 GGGGACGAAAAGTACCGCGAGGCTGCATGGAGAATGTTCACGGCTATCGAAGCGGTTACA 1595

Qy        521 LysThrGluPheGlyAsnAlaAlaValArgAspValMetValGluGluGlyAsnValLys 540
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Db       1596 AAGACGGAGTTTGGGAATGCGGCGGTGCGGGATGTTATGGTTGAGGAAGGAAATGTAAAG 1655

Qy        541 ArgGluAspSerMetGluSerPheTrpMetAlaGluThrLeuLysThrLeuThrLeuIle 560
              ||||||||||||||||||||||||||||||||||||||||||||   |||   ||||||
Db       1656 AGAGAAGATAGCATGGAGAGTTTCTGGATGGCAGAGACGTTGAAGTATCTGTATCTGATA 1715

Qy        561 PheGlyGluThrAspLeuValSerLeuAspAspTrpValPheAsnThrGluAlaHisPro 580
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Db       1716 TTTGGGGAGACCGATTTGGTCAGCTTGGACGACTGGGTGTTCAATACGGAGGCGCACCCT 1775

Qy        581 LeuArgGlyAlaGlySer 586
              ||||||||||||||||||
Db       1776 TTGAGGGGTGCAGGGAGT 1793
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APPENDIX B (GenBank Accession Number AA965900)

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RESULT 1
AA965900
LOCUS       AA965900                524 bp    mRNA    linear   EST 31-JUL-1998
DEFINITION  o8h03a1.r1 Aspergillus nidulans 24hr asexual developmental and
            vegetative cDNA lambda zap library Emericella nidulans cDNA clone
            o8h03a1 5', mRNA sequence.
ACCESSION   AA965900
VERSION     AA965900.1  GI:3139784
KEYWORDS    EST.
SOURCE      Emericella nidulans (anamorph: Aspergillus nidulans)
  ORGANISM  Emericella nidulans
            Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;
            Eurotiales; Trichocomaceae; Emericella.
REFERENCE   1  (bases 1 to 524)
  AUTHORS   Kupfer,D., Gray,J., Hausner,J., Lai,H., Martin,W., Aramayo,R.,
            Prade,R. and Roe,B.
  TITLE     An Aspergillus nidulans EST Database
  JOURNAL   Unpublished
COMMENT     Other_ESTs: o8h03a1.f1
            Contact: Bruce A. Roe, University of Oklahoma, broe@ou.edu
            Department of Chemistry and Biochemistry
            Advanced Center for Genome Technology, University of Oklahoma
            620 Parrington Oval, Norman, OK 73019, USA
            Tel: 405 325 4912
            Fax: 405 325 7762
            Email: broe@ou.edu
            We anticipate the future release of the cDNA clones to the Fungal
            Genetics Stock Center
            Seq primer: T3
            High quality sequence stop: 400.
FEATURES             Location/Qualifiers
     source          1. .524
                     /organism="Emericella nidulans"
                     /mol_type="mRNA"
                     /strain="FGSC A26"
                     /db_xref="taxon:162425"
                     /clone="o8h03a1"
                     /tissue_type="vegetative mycelia, asexual structures"
                     /clone_lib="Aspergillus nidulans 24hr asexual
                     developmental and vegetative cDNA lambda zap library"
                     /note="Vector: pBlueScript SK-; Site_1: EcoRI; Site_2:
                     XhoI; 5' end of cDNA cloned into EcoRI site of pBluescript
                     3' end of cDNA cloned into XhoI site of pBluescript"
BASE COUNT      122 a    161 c    152 g     89 t
ORIGIN

  Query Match             25.8%;  Score 524;  DB 9;  Length 524;
  Best Local Similarity   100.0%;  Pred. No. 4.8e-259;
  Matches  524;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy        371 AGCAGTCAAAGGTGCCTTTCAGCGCGCATGGACCTCCTACACAACCCACGCCTGGAAGGC 430
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 AGCAGTCAAAGGTGCCTTTCAGCGCGCATGGACCTCCTACACAACCCACGCCTGGAAGGC 60

Qy        431 GGACGAGGTACGGCCCATCACGGCCGGATCTCGAAACAACTTTGGCGGATGGGGAGCGAC 490
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 GGACGAGGTACGGCCCATCACGGCCGGATCTCGAAACAACTTTGGCGGATGGGGAGCGAC 120

Qy        491 CCTAGTCGACAATCTCGACACACTGCTAATCATGGGGCTGGACGAGGAGTTCGCAGCGGC 550
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 CCTAGTCGACAATCTCGACACACTGCTAATCATGGGGCTGGACGAGGAGTTCGCAGCGGC 180

Qy        551 AGTCGACGCGCTCGCAGATATAGAATTCAGCCCGCACTCGTCCCCATCCTCCTCCCAGAG 610
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 AGTCGACGCGCTCGCAGATATAGAATTCAGCCCGCACTCGTCCCCATCCTCCTCCCAGAG 240
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Qy        611 CACAATCAACATATTCGAAACGACAATCCGGTATCTGGGCGGCTTGCTCGCGGCGTATGA 670
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 CACAATCAACATATTCGAAACGACAATCCGGTATCTGGGCGGCTTGCTCGCGGCGTATGA 300

Qy        671 TCTCACTGGCTGTCGAGAGACTCGGCTGCTGGACAAAGCAATCCAGCTTGGGGAGATGAT 730
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 TCTCACTGGCTGTCGAGAGACTCGGCTGCTGGACAAAGCAATCCAGCTTGGGGAGATGAT 360

Qy        731 CTACACCTCCTTCGACACAGAGAACCGCATGCCCGTACCACGGTGGAATCTGCACAAAGC 790
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 CTACACCTCCTTCGACACAGAGAACCGCATGCCCGTACCACGGTGGAATCTGCACAAAGC 420

Qy        791 AGGCAACGGAGAGCCTCAGCGCGCGGCAGTGCAGGGCGTGCTCGCTGAACTCGCCAGCAG 850
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        421 AGGCAACGGAGAGCCTCAGCGCGCGGCAGTGCAGGGCGTGCTCGCTGAACTCGCCAGCAG 480

Qy        851 CAGTCTCGAGTTCACGCGGCTGTCGCAGCTGACGGGGGATATGC 894
              |||||||||||||||||||||||||||||||||||||||||||||
Db        481 CAGTCTCGAGTTCACGCGGCTGTCGCAGCTGACGGGGGATATGC 524
```

APPENDIX C (Activities Encompassed by "Mannosidase")

1) EC 3.2.1.113
Common name: mannosyl-oligosaccharide 1,2-α-mannosidase
Reaction: Hydrolysis of the terminal 1,2-linked α-D-mannose residues in the oligo-mannose oligosaccharide $Man_9(GlcNAc)_2$
Other name(s): mannosidase 1A; mannosidase 1B; 1,2-α-mannosidase; exo-α-1,2-mannanase; mannose-9 processing α-mannosidase; glycoprotein processing mannosidase I; mannosidase I; Man9-mannosidase
Systematic name: 1,2-α-mannosyl-oligosaccharide α-D-mannohydrolase

2) EC 3.2.1.114
Common name: mannosyl-oligosaccharide 1,3-1,6-α-mannosidase
Reaction: Hydrolysis of the terminal 1,3- and 1,6-linked α-D-mannose residues in the mannosyl-oligosaccharide $Man_5(GlcNAc)_3$
Other name(s): mannosidase II; exo-1,3-1,6-α-mannosidase; α-D-mannosidase II; α-mannosidase II; α1-3,6-mannosidase; GlcNAc transferase I-dependent α1,3[α1,6]mannosidase; Golgi α-mannosidase II
Systematic name: 1,3-(1,6-)mannosyl-oligosaccharide α-D-mannohydrolase

3) EC 3.2.1.24
Common name: α-mannosidase
Reaction: Hydrolysis of terminal, non-reducing α-D-mannose residues in α-D-mannosides
Systematic name: α-D-mannoside mannohydrolase
Other name(s): α-D-mannosidase; *p*-nitrophenyl-α-mannosidase; α-D-mannopyranosidase; 1,2-α-mannosidase; 1,2-α-D-mannosidase; exo-α-mannosidase

4) EC 3.2.1.130
Common name: glycoprotein endo-α-1,2-mannosidase
Reaction: Hydrolysis of the terminal α-D-glucosyl-(1,3)-D-mannosyl unit from the $GlcMan_9(GlcNAc)_2$ oligosaccharide component of the glycoprotein produced in the Golgi membrane
Other name(s): glucosylmannosidase; endo-α-D-mannosidase; endo-α-mannosidase; endomannosidase; glucosyl mannosidase
Systematic name: glycoprotein glucosylmannohydrolase

5) EC 3.2.1.77
Common name: mannan 1,2-(1,3)-α-mannosidase
Reaction: Hydrolysis of 1,2- and 1,3-linkages in yeast mannan, releasing mannose
Other name(s): exo-1,2-1,3-α-mannosidase
Systematic name: 1,2-1,3-α-D-mannan mannohydrolase

6) EC 3.2.1.25
Common name: β-mannosidase
Reaction: Hydrolysis of terminal, non-reducing β-D-mannose residues in β-D-mannosides
Other name(s): mannanase; mannase; β-D-mannosidase; β-mannoside mannohydrolase; exo-β-D-mannanase
Systematic name: β-D-mannoside mannohydrolase

7) EC 3.2.1.137
Common name: mannan exo-1,2-1,6-α-mannosidase
Reaction: Hydrolysis of 1,2-α-D- and 1,6-α-D- linkages in yeast mannan, releasing D-mannose
Other name(s): exo-1,2-1,6-α-mannosidase
Systematic name: 1,2-1,6-α-D-mannan D-mannohydrolase

8) EC 3.2.1.101
Common name: mannan endo-1,6-α-mannosidase
Reaction: Random hydrolysis of 1,6-α-D-mannosidic linkages in unbranched 1,6-mannans
Other name(s): exo-1,6-β-mannanase; endo-α-1→6-D-mannanase; endo-1,6-β-mannanase; mannan endo-1,6-β-mannosidase
Systematic name: 1,6-β-D-mannan mannanohydrolase

9) EC 3.2.1.78
Common name: mannan endo-1,4-β-mannosidase
Reaction: Random hydrolysis of 1,4-β-D-mannosidic linkages in mannans, galactomannans and glucomannans
Other name(s): endo-1,4-β-mannanase; endo-β-1,4-mannase; β-mannanase B; β-1, 4-mannan 4-mannanohydrolase; endo-β-mannanase; β-D-mannanase
Systematic name: 1,4-β-D-mannan mannanohydrolase